Talanta 77 (2009) 1149–1154

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

A microband lactate biosensor fabricated using a water-based screen-printed carbon ink

F.J. Rawson[a], W.M. Purcell[b], J. Xu[c], R.M. Pemberton[a], P.R. Fielden[d], N. Biddle[e], J.P. Hart[a,*]

[a] Centre for Research in Analytical, Materials and Sensors Science, Faculty of Health and Life Sciences, University of the West of England, Bristol, UK
[b] University of Plymouth, Drake Circus, Plymouth, UK
[c] Centre for Research in Biomedicine, Faculty of Health and Life Sciences, University of the West of England, Bristol, UK
[d] School of Chemical Engineering and Analytical Science, Faculty of Engineering and Physical Sciences, The University of Manchester, Manchester, UK
[e] Gwent Electronic Materials Ltd., Monmouth House, Mamhilad Park, Pontypool, UK

ARTICLE INFO

Article history:
Received 6 June 2008
Received in revised form 6 August 2008
Accepted 18 August 2008
Available online 3 September 2008

Keywords:
Lactate
Microband biosensors
Hydrogen peroxide
Screen-printing
Cobalt phthalocyanine

ABSTRACT

The present study demonstrated for the first time that screen-printed carbon microband electrodes fabricated from water-based ink can readily detect H_2O_2 and that the same ink, with the addition of lactate oxidase, can be used to construct microband biosensors to measure lactate. These microband devices were fabricated by a simple cutting procedure using conventional sized screen-printed carbon electrodes (SPCEs) containing the electrocatalyst cobalt phthalocyanine (CoPC). These devices were characterised with H_2O_2 using several electrochemical techniques. Cyclic voltammograms were found to be sigmoidal; a current density value of 4.2 mA cm^{-2} was obtained. A scan rate study revealed that the mass transport mechanism was a mixture of radial and planar diffusion. However, a further amperometric study under quiescent and hydrodynamic conditions indicated that radial diffusion predominated. A chronoamperometric study indicated that steady-state currents were obtained with these devices for a variety of H_2O_2 concentrations and that the currents were proportional to the analyte concentration. Lactate microband biosensors were then fabricated by incorporating lactate oxidase into the water-based formulation prior to printing and then cutting as described. Voltammograms demonstrated that lactate oxidase did not compromise the integrity of the electrode for H_2O_2 detection. A potential of +400 mV was selected for a calibration study, which showed that lactate could be measured over a dynamic range of 1–10 mM which was linear up to 6 mM; a calculated lower limit of detection of 289 μM was ascertained. This study provides a platform for monitoring cell metabolism *in-vitro* by measuring lactate electrochemically via a microband biosensor.

1. Introduction

Screen-printing technology has been used extensively to develop biosensors for important biomedical markers [1]. Lactate is an important marker in cell metabolism and the purpose of the current study was to develop an electrochemical biosensor to monitor this metabolite. In order to monitor cell metabolism for a 24 h period it is necessary to produce microband biosensors to avoid any significant perturbation of the system and avoid any adverse effects on the cells. Microelectrodes can be formed by a variety of methods including laser micromachining [2], sonoelectrochemistry [3,4] and photolithography techniques [5]; and by direct cutting as shown by Chang and Zen [6] and Authier et al. [7]. The method of Authier et al. and one previously reported by our group [8] are considered to be the simplest to use and a similar method was used in the present study to produce microband electrodes. Microband electrodes have several advantages over conventional sized electrodes (mm^2) including low ohmic drop, steady-state currents, independence of signal on stir rate and increased signal-to-noise ratios, leading to lower detection limits [9,10].

In this investigation, microband biosensors were fabricated using screen-printed carbon electrodes (SPCEs) produced from a water-based carbon ink. This ink has key advantages over an organic-based ink, as it is less toxic, and does not denature enzymes; this allows for a one-step printing process which greatly reduces the cost of production. This approach has been used successfully to produce glucose biosensors in which glucose oxidase was incorporated into a water-based ink and printed in a one-step print process [11,12]. In the present study, the enzyme lactate oxidase was incorporated directly into the water-based ink, together with the electrocatalyst cobalt phthalocyanine (CoPC).

* Corresponding author. Tel.: +44 1173282469.
E-mail address: john.hart@uwe.ac.uk (J.P. Hart).

0039-9140/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2008.08.020

Lactate oxidase catalyses the oxidation of lactate to pyruvate with the production of hydrogen peroxide (H_2O_2). This H_2O_2 can then be followed electrochemically via its electrocatalytic oxidation by CoPC; the reaction scheme can be seen in Eqs. (1) and (2). The reaction mechanism for this process has been previously reported by our group [13,14].

$$2Co^{2+}PC + H_2O_2 \rightarrow 2Co^{+}PC + 2H^{+} + O_2 \quad (1)$$

$$2Co^{+}PC \rightarrow 2Co^{2+}PC + 2e^{-} \quad (2)$$

This study demonstrates for the first time that screen-printed carbon microband electrodes, fabricated from water-based ink can readily detect H_2O_2 and that the same ink, with the addition of lactate oxidase, can be used to construct biosensors for lactate measurements. To our knowledge, this is the first report of a microband biosensor for lactate measurements using such an approach.

2. Experimental

2.1. Chemicals and reagents

All chemicals were purchased from Sigma–Aldrich. A 0.5 M sodium lactate stock solution in 0.25 M phosphate buffer pH 7.3 was made up fresh on each experimental day. The supporting electrolyte was phosphate buffer prepared at 0.25 M by combining appropriate amounts of di-sodium hydrogen orthophosphate and sodium dihydrogen orthophosphate. A stock solution of 0.5 M H_2O_2 solution was made up fresh in 0.25 M phosphate buffer pH 7.3 and any further concentrations of H_2O_2 were prepared via dilution of the stock with phosphate buffer. The lactate oxidase was from *Aerococcus viridans* obtained from Genzyme UK (code 1381).

2.2. Apparatus

All electrochemical measurements were carried out using a dual screen-printed electrode design. A CoPC-SPCE based on an ink code C207070501R2 containing no lactate oxidase and ink code C20727D3 which incorporated the enzyme lactate oxidase (microband lactate biosensors) was used to screen-print electrodes (Gwent Electronic Materials Ltd. (GEM)). These inks were screen-printed on one side of the 0.5 mm thick PVC substrate and a defined area of 9 mm^2 was created using dielectric tape; an Ag/AgCl reference electrode was printed around the working electrode (conventional sized Fig. 1A). Microbands were formed by cutting through the working electrode and insulator layer using scissors, thus exposing the edge of the electrodes (Fig. 1B). The geometrical area of the edge was calculated to be 5.28×10^{-4} cm^2 by multiplying the length (l) by the width (w). The value of w (17.6 μm) was measured using a TESA digital micrometer obtained from Radio Spares, Switzerland. The SPCEs were connected to the potentiostat by two gold clips attached to the ends of two electrical leads. All electrochemical measurements were made with an Autolab Pstat10 potentiostat (Windsor Scientific, Slough) and accompanying software. The electrochemical cell was a Metrohm tapered-wall glass cell with water jacket. The temperature was fixed at 25 °C by means of a circulating water bath (HAAKE D3).

2.3. Cyclic voltammetric measurements of hydrogen peroxide at microband SPCEs

Cyclic voltammograms were obtained by scanning from 0 to +1 V at a scan rate of 20 mV s^{-1} using a 10 ml aliquot of 5 mM H_2O_2. This was done using both plain microband SPCEs which did not contain the electrocatalyst CoPC and microband SPCEs that contained the electrocatalyst (CoPC-SPCEs). This was performed to show that

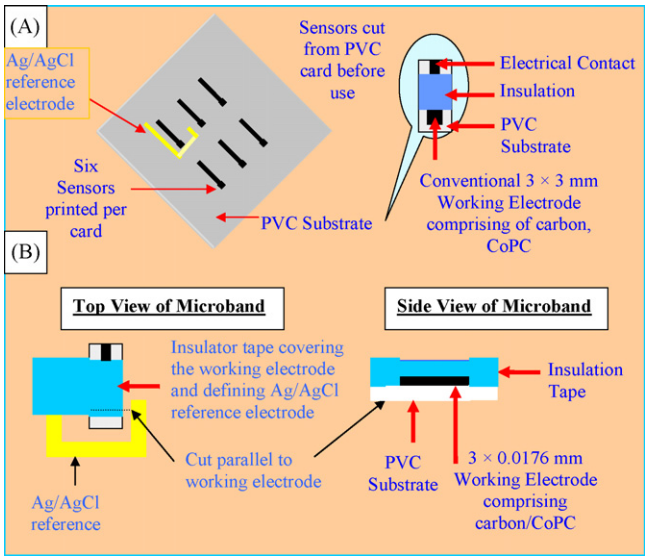

Fig. 1. Schemic diagram of SPCEs used and method to produce (A) conventional and (B) microband electrodes.

the signal generated occurred via the electrocatalytic oxidation of H_2O_2. A scan rate study using 1, 10, 20 and 50 mV s^{-1} for H_2O_2 was carried out to establish whether microelectrode behaviour occurred as indicated by the appearance of steady-state currents [9].

2.4. Cyclic voltammetric measurements of lactate using microband CoPC-SPCEs and microband biosensors

Cyclic voltammograms were obtained by scanning from 0 to +1 V at a scan rate of 20 mV s^{-1} using a 10 ml aliquot of 5 mM lactate solution. These voltammograms were generated at, microband CoPC-SPCEs without lactate oxidase, and microband biosensors containing both CoPC and lactate oxidase (microband biosensors).

2.5. Effect of stirring on the amperometric response for H_2O_2 using microband CoPC-SPCEs

Amperometry was used to obtain current versus potential plots in quiescent and stirred solution containing 5 mM H_2O_2 and blank 0.25 M pH 7.3 phosphate buffer solutions. This was done by transferring a 10 ml aliquot of H_2O_2 solution or blank solution to the cell, stepping the potential from open circuit to 0 V, then stepping from 0 to +0.1 V and continuing in this way to a final potential of +1 V. The voltammograms constructed using the mean value from triplicate measurements were used to ascertain the effect of stirring on the response and also, to deduce the potential to be used in later amperometric studies.

2.6. Calibration study on H_2O_2 using chronoamperometry with microband CoPC-SPCEs

Standard solutions containing 0.07, 0.21, 0.7, 2.1 and 7 mM H_2O_2 were prepared in 0.25 M pH 7.3 phosphate buffer. Chronoamperograms were then recorded using a fresh microband CoPC-SPCE and 10 ml aliquots of each H_2O_2 solution. Each solution was examined by inserting the electrode and leaving at open circuit for 5 s before applying a potential of +400 mV for a period of 100 s. One electrode was used for all solutions examined, and this procedure repeated using a further four electrodes to give a total of five replicate measurements at each concentration.

2.7. Optimisation of the potential for amperometric measurements of lactate using microband biosensors

In order to deduce the optimum applied potential for calibration studies, amperometric measurements were made at various potentials in lactate and blank buffer solutions. The microband biosensors were placed in 5 mM lactate solution for 500 s for the first two potential steps then 2000 s for the succeeding application of each potential step up to +1 V. The resulting data was used to plot a voltammogram from which the potential to be used in amperometric studies was deduced. It should be added that the time taken to reach a steady current is considerably less at the lower potentials, i.e. below +200 mV; consequently shorter analysis times are required for the current data sets in this region.

2.8. The effect of pH on the response generated at microband biosensors for lactate determination

The effect of pH was investigated over the pH range 6–8 at a constant phosphate buffer strength of 0.25 M with microband biosensors. A calibration study was performed by adding 40 μl aliquots of a 0.25 M solution of lactate to a 10 ml solution of 0.25 M phosphate buffer giving 1 mM additions of lactate. A fixed potential of +400 mV versus a Ag/AgCl reference electrode was used throughout the study under quiescent conditions.

2.9. The Effect of buffer strength on the response generated at microband biosensors for lactate determination

The effect of buffer strength over the range 0.1, 0.25, and 0.5 M pH 7.3 phosphate buffer was investigated with microband biosensors. Cyclic voltammograms were obtained by scanning from 0 to +1 V at a scan rate of 20 mV s^{-1}, using a 10 ml aliquot of 5 mM lactate in the different buffer; blank buffers without the lactate present were also examined under the same conditions. This was done in triplicate using a fresh electrode for each voltammogram.

2.10. Calibration study using amperometry with microband biosensors for lactate detection

Calibration studies were carried out with microband biosensors by making 40 μl additions of a 0.25 M solution of lactate to a 10 ml solution of 0.25 M pH 7.3 phosphate buffer containing 0.1 M NaCl equating to 1 mM additions of lactate. Amperograms were recorded using a fresh microband biosensor for each calibration curve. After switching to +400 mV for a period 1000 s, to ensure that a steady-state was reached, lactate additions were made every 300 s up to a 10 mM lactate concentration.

3. Results and discussion

3.1. Cyclic voltammetric measurements of hydrogen peroxide at microband CoPC-SPCEs and plain microband SPCEs

An initial cyclic voltammetric study was performed to establish whether H_2O_2 could be measured using the new microband CoPC-SPCEs. The voltammogram is shown in Fig. 2IA for H_2O_2 at this electrode and clearly exhibits an electrocatalytic oxidation signal at approximately +0.5 V (Fig. 2I(A); the lack of a peak on the voltammogram obtained in the plain buffer solution (Fig. 2I(B)) demonstrates that H_2O_2 is responsible for the response [8]. We also carried out the equivalent experiment but with microband SPCEs that contained no electrocatalyst; clearly no signal for H_2O_2 was observed (Fig. 2I(C)). From these results it can be deduced

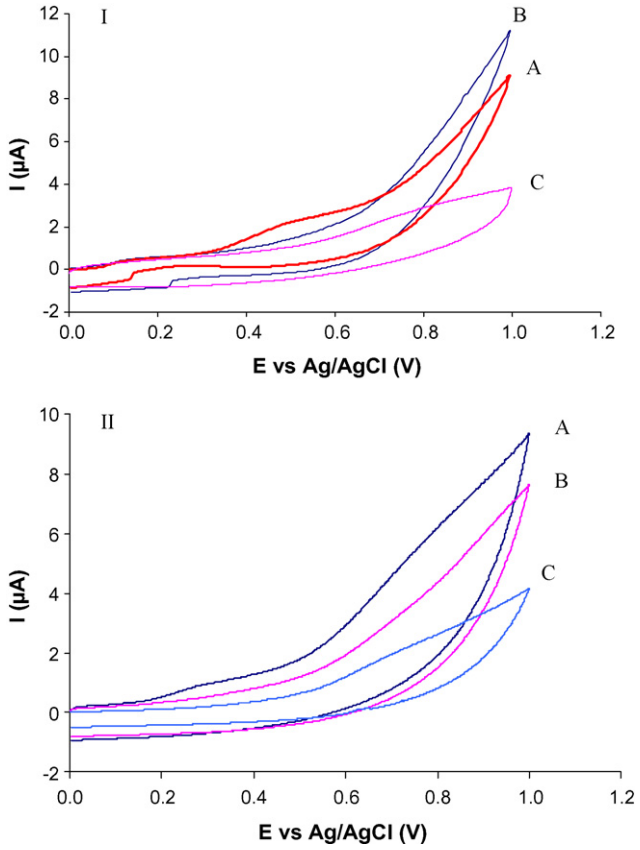

Fig. 2. (I) Typical cyclic voltammogram obtained using a microband CoPC-SPCE (A and B): for 5 mM H_2O_2 (A) and plain 0.25 M phosphate buffer pH 7.3 (B). A typical voltammogram obtained using microband SPCEs (no electrocatalyst) for 5 mM H_2O_2 (C). (II) Typical cyclic voltammograms obtained for 5 mM lactate using microband biosensors (A and B): for 5 mM lactate (A) and plain 0.25 M phosphate buffer pH 7.3 (B). Typical voltammograms are also shown obtained using microband CoPC-SPCEs (C) that contained no enzyme obtained for 5 mM lactate.

that H_2O_2 is catalytically oxidised through Co^{2+}/Co^{+} redox couple [8,14]. It is also interesting to note that the new design microband CoPC-SPCEs gave a current density value calculated from Fig. 2I(A) of 4.2 mA cm^{-2} compared to 290 μA cm^{-2} for conventional sized CoPC-SPCEs; this is due to the more efficient mass transport at the microband electrode surface, where radial diffusion is occurring, compared to the planar diffusion which is seen at the conventional sized electrode surface. Such high current density values have been seen previously for screen-printed tubular microband electrodes [8].

A scan rate study was performed with the new microband electrodes and the resulting voltammograms and steady-state currents are plotted in Fig. 3. Voltammograms in Fig. 3A show steady-state currents are obtained for the electrocatalytic oxidation of hydrogen peroxide at a CoPC-SPCE microband electrode over the range 1–50 mV $s^{-1.}$ This behaviour is indicative of mass transport by radial diffusion. This supports our earlier conclusion regarding the mechanism of diffusion control, where current density of 4.2 mA cm^{-2} was calculated compared to 290 μA cm^{-2} seen for a conventional sized electrode. The plot of mean steady-state currents (Fig. 3B) suggests that mass transport does not occur by planar diffusion as currents would be expected to increase with the square root of scan rate. However, the signals do not appear to be completely independent of scan rate, which may indicate a mixed mechanism of mass transport involving both radial and planar diffusion.

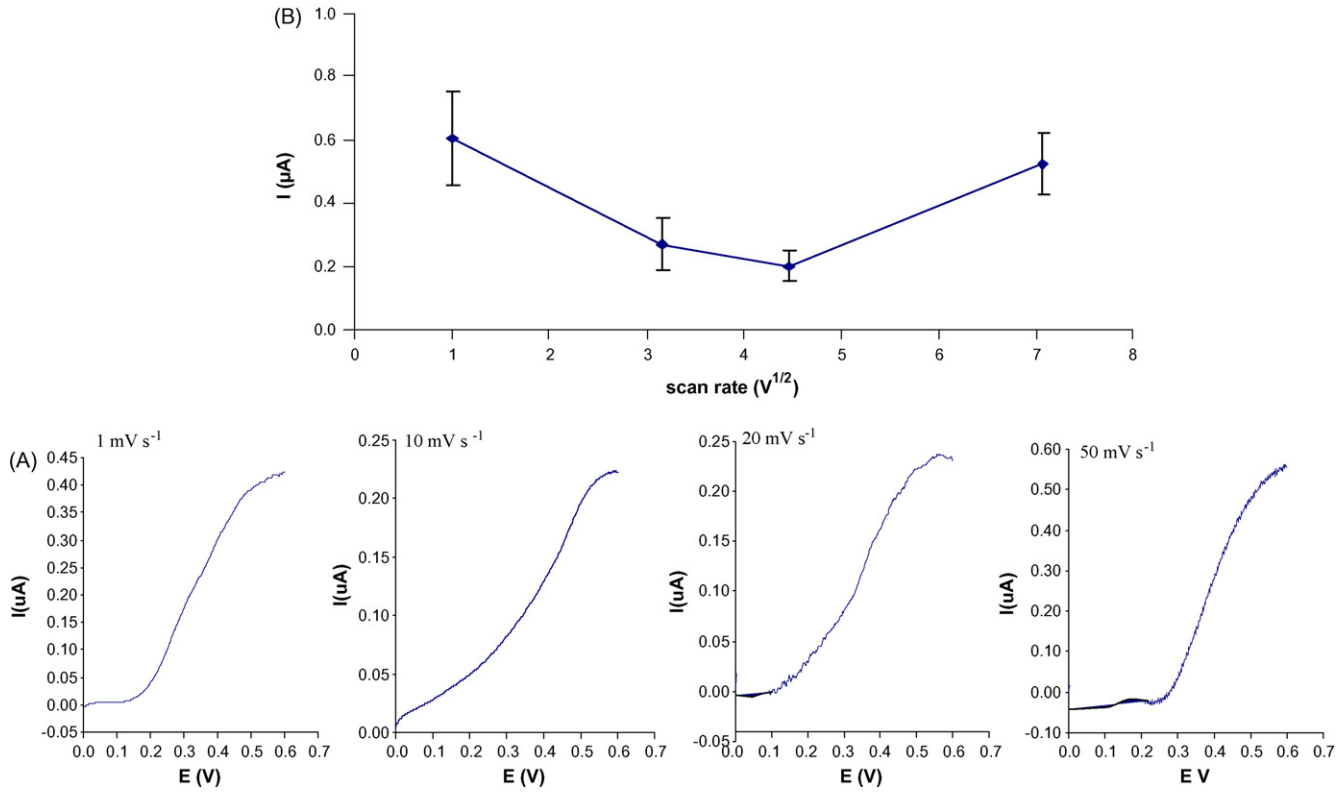

Fig. 3. Typical voltammograms (A) and mean steady-state currents (B) obtained at various scan rates: 1, 10, 20 and 50 mV s^{-1} for 5 mM $H_2O_2 \pm 1$ S.D. ($n = 3$).

3.2. Effects of stirring on the amperometric response for H_2O_2

In order to further examine the mass transport behaviour of H_2O_2 at a microband CoPC-SPCE, amperometric studies were performed under quiescent and hydrodynamic conditions over the potential range +0.1 to +1 V. The resulting voltammograms are shown in Fig. 4. It can be seen that there is no significant difference in the maximum currents observed at around +0.5 V. This confirms that radial diffusion is the predominant mechanism of H_2O_2 transport to the electrode.

3.3. Calibration study on H_2O_2 using chronoamperometry with microband CoPC-SPCEs

The next stage of the investigation was to establish whether these microband electrodes could form the basis for the proposed lactate biosensors and an applied potential of +0.4 V was used in this study. It should be mentioned that this potential was selected for calibration rather than the potential where current is at a maximum for two reasons: first, less interference will occur at the lower potentials; second, we wish to avoid the possibility of the irreversible reaction which is known to occur with this electrocatalyst at potentials around +0.5 to +0.6 V. This reaction results in a loss of sensitivity over time and has been reported previously [8,14]. Fig. 5 shows the chronoamperograms obtained for several different concentrations of H_2O_2 and it is apparent that steady-state currents were obtained with these devices, as is expected when radial diffusion predominates. The magnitude of the signals was shown to be directly proportional to the concentration of H_2O_2 over the range

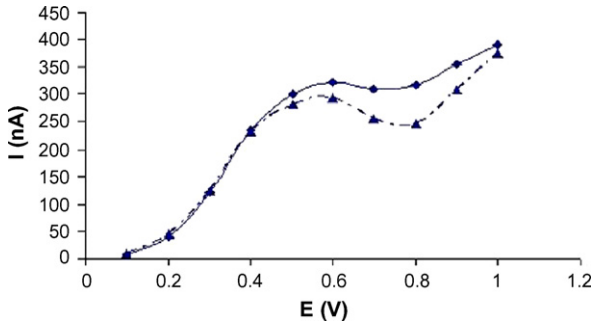

Fig. 4. Voltammograms for 5 mM H_2O_2 obtained by stepping the potential from open circuit voltage in +100 mV steps in stirred (♦) and quiescent (▲) solution using microband CoPC-SPCE electrodes. The supporting electrolyte was 0.25 M phosphate buffer at pH 7.3 and blank voltammograms have been subtracted in each case.

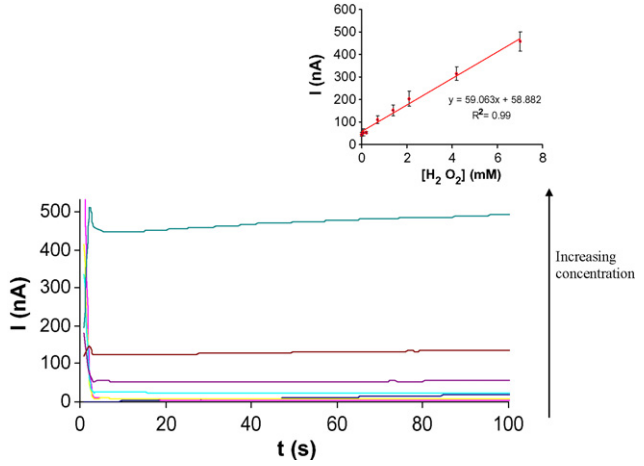

Fig. 5. Typical chronoamperograms obtained for various H_2O_2 concentrations and calibration graph (inset) obtained using an applied potential of +400 mV error bars ± 1 S.D. ($n = 5$).

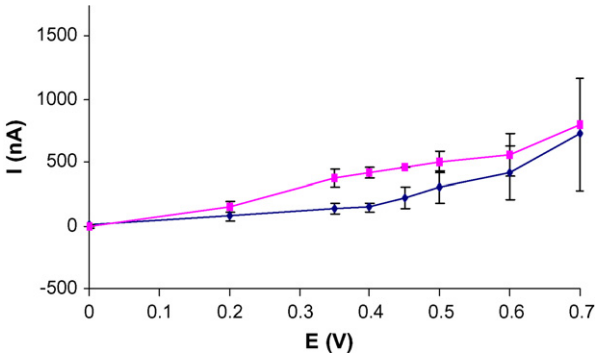

Fig. 6. Voltammograms obtained by stepping the potential from open circuit voltage in +100 mV steps in 5 mM lactate (■) or buffer solution (▲) using microband lactate biosensors.

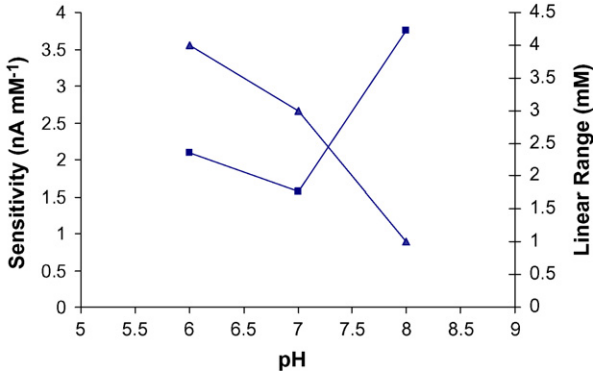

Fig. 7. The effect of pH on microband biosensor performance, showing both lactate sensitivity (squares) and linear range (triangles). Data obtained from amperograms obtained from fixed potential current curves obtained using standard additions of 1 mM lactate.

studied as seen in the inset in Fig. 5. From this plot the linear range was deduced to be 0.07–7 mM H_2O_2, with a sensitivity value of 59 nA mM^{-1} and the theoretical limit of detection calculated via twice the standard deviation of the noise (0.44 nA) divided by the sensitivity gave a lower limit of 15 μM. Typical coefficients of variation were obtained in the range 9–18.5%. These results indicated that the devices could readily form the basis of a lactate microband biosensor.

3.4. Cyclic voltammetric measurements of lactate at microband CoPC-SPCEs and microband biosensors

Cyclic voltammograms were obtained with a 5 mM lactate solution and blank pH 7.3 0.25 M phosphate buffer using microband lactate biosensors fabricated from WB-CoPC-SPCEs. An oxidation signal for lactate was seen at +300 mV (Fig. 2II(A)) that is not apparent in the blank buffer solution (Fig. 2II(B)). Cyclic voltammograms were also obtained for lactate with microband CoPC-SPCEs without enzyme; the resulting voltammogram did not show a peak at +300 mV (Fig. 2II(C)). These results demonstrate that it is not possible to detect lactate by direct oxidation at the CoPC-SPCE but that it is readily measured with a microband lactate biosensor; the latter operates by the detection of H_2O_2 produced by lactate oxidase.

3.5. Optimisation of applied potential for the lactate microband biosensor

Fig. 6 shows the voltammograms obtained with the microband biosensor using a 5 mM lactate solution at pH 7.3 or plain buffer solutions. As mentioned earlier, the lactate response results from the electrocatalytic oxidation of H_2O_2 which is enzymatically produced during the enzymatic oxidation of lactate to pyruvate by the lactate oxidase. The voltammetric profile is similar to that obtained for H_2O_2 with plain microband CoPC-SPCEs (Fig. 4). This indicates that there is no significant effect on the response when the enzyme is incorporated into the ink. For analytical purposes a potential of +400 mV was selected for calibration studies.

3.6. The effect of pH and buffer strength on the current response generated at microband biosensors for lactate determination

The effect of pH on biosensor performance can be seen in Fig. 7; clearly maximum sensitivity was obtained at pH 8 but the largest linear range was obtained at pH 6. pH 7 offered intermediate linear range and sensitivity. This is perhaps expected, as the optimum has been reported to be pH 6.4 [15]. Although this behaviour is interesting, our proposed application for microband biosensors is to monitor cell metabolism in culture medium of pH 7.3. Consequently, we have selected pH 7.3 for further studies. However, for some applications which do not involve live cells such as milk analysis, a biosensor operating at a pH of 6 may be of benefit. The effect of buffer strength on the steady-state current generated for cyclic voltammograms obtained with 5 mM lactate solution can be seen in Table 1. The buffer strength that yielded the highest steady-state currents was 0.25 M phosphate buffer; this concentration was used in further studies.

Table 1
Table of steady-state currents obtained for cyclic voltammograms recorded in 5 mM lactate solutions at different ionic strengths

	Ionic strength of phosphate buffer (M)		
	0.125	0.25	0.5
Ip (μA)	0.248 0.322 0.348	1.518 1.289 1.287	0.12 0.13 0.1
Mean	0.31	1.36	0.12
Standard deviation	0.05	0.13	0.02
Coefficients of variation (%)	16.9	9.7	13.1

3.7. Calibration studies for lactate at microband screen-printed biosensors

Fig. 8 shows a typical amperometric response for lactate additions to a microband biosensor immersed in 0.25 M pH 7.3 phosphate buffer solution. The additions correspond to added con-

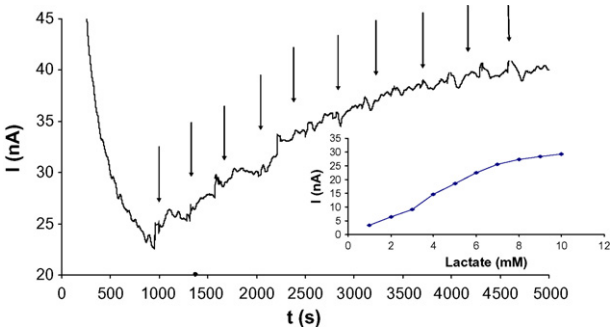

Fig. 8. Typical amperometric responses obtained for standard lactate additions using an applied potential of +400 mV with microband lactate biosensors and the resulting calibration graph (each data point is the mean of current value obtained at five calibration studies).

centrations of 1 mM. It is evident that, even though solutions were not stirred, the responses to lactate were reasonably rapid and steady-state currents were obtained; lactate could be measured over the range 1–10 mM. A linear range was obtained up to 6 mM lactate ($y = 3.6258x$) giving a R^2 value of 0.98 and an intra-electrode coefficient of variation over this range of 9% ($n = 6$). A sensitivity value over this range of 3.63 nA mM^{-1} was obtained with a theoretical lower detection limit of 289 μM based on three times the mean noise. The sensors were stable for up to two weeks. These results compare relatively well to other published data on macro-sized biosensors incorporating lactate oxidase to detect lactate with values recorded recently in the literature of 4.4 μM [16], 10 μM [17], and 300 μM [18].

4. Conclusions

It has been shown that microband SPCEs fabricated using a water-based screen-printing ink incorporating CoPC gave a well-defined electrocatalytic response for H_2O_2. The resulting voltammogram was similar to that previously described for a tubular microband electrode, fabricated using an organic-based carbon ink [8]. Evidence that our electrodes behave as microband devices may be deduced by the higher current density compared to conventional planar screen-printed electrodes. This behaviour suggests that a significant proportion of the current is generated by radial diffusion. Further evidence to support this diffusion behaviour was demonstrated by the observation that the voltammetric response did not increase proportionally with the square root of scan rate. The microband CoPC-SPCEs could be used to detect H_2O_2 over a wide concentration range from 70 to 7000 μM and significantly, steady-state currents were obtained by chronoamperometry.

It was demonstrated that our microband lactate biosensor could readily measure the analyte over a linear range 1–6 mM (Fig. 8). This study represents the first report on the manufacture and use of microband biosensors based on screen-printing technology for lactate determination. These lactate microband biosensors will form the platform for future work in measuring cell metabolism, via the release of lactate, in quiescent solutions over extended incubation times.

Acknowledgements

The authors wish to thank GEM (Gwent Electronic Materials Ltd.) for their support in providing the electrode materials. They are also grateful to the University of the West of England for continued support and providing funds to carry out the research.

References

[1] J.P. Hart, A. Crew, E. Crouch, K.C. Honeychurch, R.M. Pemberton, in: S. Alegret, A. Merkoci (Eds.), Comprehensive Analytical Chemistry, vol. 49, 2007, p. 497 (Chapter 23).
[2] C. Ball, D.L. Scott, J.K. Lump, S. Daunert, J. Wang, L.G. Bachas, Anal. Chem. 72 (2000) 497.
[3] K.A. Law, S.P.J. Higson, Biosens. Bioelectron. 20 (2005) 1914.
[4] J. Pritchard, K. Law, A. Vakurvov, P. Millner, S.P.J. Higson, Biosens. Bioelectron. 20 (2004) 765.
[5] J. Burmeister, M. Palmer, G.A. Gerhardt, Biosens. Bioelectron. 20 (2005) 1772.
[6] J.-L. Chang, J.-M. Zen, Electrochem. Commun. 8 (2006) 571.
[7] L. Authier, C. Groissiord, P. Brossier, Anal. Chem. 73 (2001) 4450.
[8] F.J. Rawson, W.M. Purcell, J. Xu, D.C. Cowell, P.R. Fielden, N. Biddle, J.P. Hart, Electrochim. Acta 52 (2007) 7248.
[9] X. Xie, D. Stueben, Z. Berner, Anal. Lett. 38 (2005) 2281.
[10] A.M. Bond, Analyst 119 (1994) R1.
[11] E. Crouch, D.C. Cowell, S. Hoskins, R.W. Pittson, J.P. Hart, Anal. Biochem. 347 (2005) 17.
[12] E. Crouch, D.C. Cowell, S. Hoskins, R.W. Pittson, J.P. Hart, Biosens. Bioelectron. 21 (2005) 712.
[13] J.P. Hart, A. Crew, E. Crouch, K.C. Honeychruch, R.M. Pemberton, Anal. Lett. 37 (2004) 789.
[14] M.A.T. Gilmartin, R.J. Ewen, J.P. Hart, C.L. Honeybourne, Electroanalysis 7 (1995) 547.
[15] J. Huang, J. Li, Y. Yang, X. Wang, B. Wu, J. Anzai, T. Osa, Q. Chen, Mater. Sci. Eng. 28 (2007) 1071.
[16] D. Lowinsohn, M. Bertotti, Anal. Biochem. 365 (2007) 260.
[17] F. Ghamouss, S. Ledru, N. Ruille, F. lantier, M. Boujtita, Anal. Chim. Acta 570 (2006) 158.
[18] N. Sato, H. Okuma, Anal. Chim. Acta 565 (2006) 250.